



(12)

EUROPEAN PATENT APPLICATION

(21) Application number: 88118412.1

(51) Int. Cl. 4: G01N 15/14, G05D 16/20

(22) Date of filing: 04.11.88

(30) Priority: 25.11.87 US 125095

(43) Date of publication of application:
31.05.89 Bulletin 89/22

(64) Designated Contracting States:
AT BE CH DE ES FR GB GR IT LI NL SE

(71) Applicant: Becton Dickinson and Company
One Becton Drive
Franklin Lakes New Jersey 07417-1880(US)

(72) Inventor: North, Howard L., Jr.
100 Via Santa Maria
Los Gatos California(US)

(74) Representative: Selting, Günther, Dipl.-Ing. et al
Patentanwälte von Kreisler, Selting, Werner
Delchmannhaus am Hauptbahnhof
D-5000 Köln 1(DE)

(54) Sheated particle flow controlled by differential pressure.

(57) A flow apparatus for the analysis of particles passing substantially one at a time through an analysis region includes a flow rate control responsive to changes in the pressure used to drive the particles through the analysis region. The flow rate control automatically regulates the flow to a preset value throughout the analysis by adjusting the particle driving pressure to be uniform even though the sheath liquid supply is depleted during the analysis. A method for controlling the flow of a supply of particles to be analyzed includes steps of sensing differential pressure and regulating the differential pressure to a preset reference.

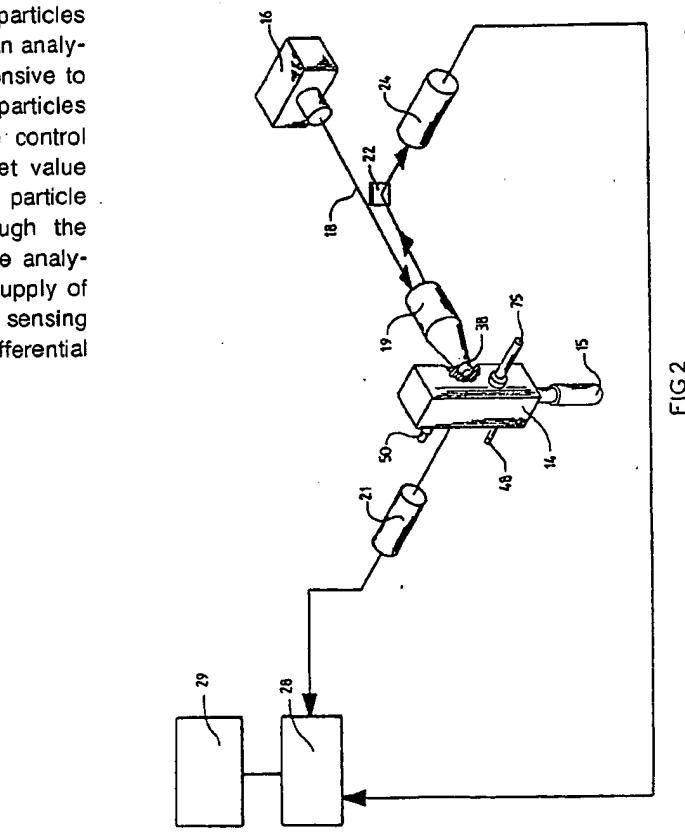


FIG 2

SHEATHED PARTICLE FLOW CONTROLLED BY DIFFERENTIAL PRESSURE

BACKGROUND OF THE INVENTIONField of the Invention.

The present invention relates to an automatic differential pressure control for a particle flow-through apparatus which includes an electronic pressure monitoring feature, and more particularly, concerns a flow cytometer for determining one or more characteristics of particles flowing with such an automatic pressure control wherein the flow rate of ensheathed particles can be regulated and maintained at a preset level to facilitate the uniform operation of the apparatus. The method of using a technique to automatically control particle flow is also a part of the present invention.

Description of the Prior Art.

There are a number of cell or particle analyzing devices using flow cytometer equipment and techniques which rely on hydrodynamically focused fluid flow through an analysis orifice where the specific characteristics of the flowing cells or particles can be determined. Flow analysis of particles has been used in the determination of the variety of characteristics of individual particles. This analysis is most useful in determining characteristics of cells for the collection of information which would aid in areas of research, hematology, immunology and the like. The researcher, for example, could be interested in determining specific characteristics of the individual cells where those cells need to be classified, identified, quantified and perhaps sorted for further investigations or analysis.

One commercially available flow cytometer which relies on a hydrodynamically focused fluid system is known as the FACScan instrument sold by the FACS Systems Division of Becton, Dickinson and Company, Mt. View, California. The FACScan instrument rapidly analyzes cells on the basis of fluorescence and volume properties. Analysis is accomplished by introducing cells in suspension to the center of a focused liquid stream and causing them to pass, one at a time, through a focused light from a high power lamp or laser. Each cell is individually characterized by its light scatter signals and by the intensity and color of fluorescence emitted while it is illuminated. Such an instrument is described in European Patent No. 0068404.

In the aforementioned flow cytometer, a sheath

liquid focuses the particles or cells as they pass through the orifice associated with the analyzing or counting capabilities. U.S. Patent Nos. 4,503,385 and 4,526,276 describe particle analysis systems in which particles flowing in a stream are enveloped in a sheath liquid which focuses and confines the sample liquid (with the particles or cells) to the center of the flowing stream. U.S. Patent No. 4,110,604 describes a particle density measuring system in which particles flowing in a stream are enveloped in a sheath liquid which focuses and confines the sample fluid (with the particles) to the center of the flowing stream.

Early systems provided for independent regulation of the air pressure used to drive the liquid with sample particles or cells from a supply test tube and of the air pressure in the sheathing liquid supply reservoir. The independent manual regulation and separate control of these two air pressures did not overcome the errors and disturbances produced by changes in liquid level in the sheathing liquid supply reservoir and pressure drop at the sheath liquid filter as it became partially obstructed with contamination or air. The air pressure regulator for the sheathing liquid supply and the other regulator for the sample test tube are required to be manually controlled. However, if reservoirs are depleted the air pressure generating the flow must be increased to maintain the proper flow rate. If the air pressure does not maintain the particle flow through the analysis orifice, the operation of the cytometer is impaired.

In the presently known and available particle flow-through equipment, electrically operated pumps, syringe pumps or the like are used in the fluidics of the system to move the liquid and particle flow through the flowcell analysis orifice and passageways. The usual operation for these pumps is to force or draw liquid with particles from a sample test tube through a sample capillary tube centered in the sheathing liquid flowing in the direction of the particle analysis orifice. These syringe pumps used to aspirate and supply the sample to the analysis orifice tend to produce carryover, washout and other problems.

With the foregoing in mind, improved techniques for overcoming pressure differential variation in particle flow-through equipment are still being sought. Such improvements in a particle flow control should preferably be included in the particle flow-through apparatus so that the various parts thereof do not have to be constantly adjusted as the sheathing liquid reservoir is depleted. It is toward such an improvement that the present invention is directed.

SUMMARY OF THE INVENTION:

The automatic differential pressure control automatically regulates and maintains the air pressure used to drive the sample liquid with particles from the test tube and can also, if needed, regulate the air pressure used to force the sheathing liquid from its reservoir. By controlling the differential pressure between the test tube and sheathing liquid pressures, uniform particle flow rates are achieved. The air pressure for driving the liquid with sample particles through the capillary tube is sensed at the pressurizing air inlet for the sample test tube. The sheathing liquid pressure is also monitored at the inlet to the flow analysis apparatus. A differential pressure transducer is connected across the aforesaid inlets of the particle flow-through apparatus of the present invention.

The apparatus has a housing with a body member having a passageway therethrough including an analysis region through which substantially one particle at a time passes in the direction of flow during operation. The differential pressure transducer provides a signal to regulate the pressure in the sample test tube for overcoming changes due to depletion of supply and to filter clogging. The object being to maintain a preset particle flow rate through the analysis orifice.

In a preferred embodiment of this aspect of the invention, the housing is suitable for a flow cytometer and includes a body member having a passageway therethrough for the passage of particles or cells which are to be analyzed. The passageway includes a pre-analysis portion, an analysis portion and a post-analysis portion aligned in that order along the axis of the passageway in that direction of flow. An air pressure supply is used for the sample test tube and another for the sheathing liquid reservoir. A differential pressure transducer is in fluid communication with the sheathing liquid inlet to the pre-analysis portion of the passageway and is also in fluid communication with the inlet of the pressurizing air for the sample test tube. The differential pressure transducer provides a varying electrical signal proportional to the monitored pressure differences. A comparator, which receives that signal and a preset reference, provides an operative control output relative to the difference therebetween. A regulator connected to receive that control output maintains the pressure differential at a preset level even though the transduced pressures vary independently of one another. The variation in transduced particle flow differential pressure is more important to the proper operation of the apparatus and is, therefore, the regulated parameter.

In another aspect of the present invention, a flow cytometer is used for determining one or more

characteristics of particles or the like flowing in a liquid stream. A body member has a passageway therethrough including an analysis region through which the moving particles pass substantially one at a time in the direction of flow. Differential pressure transducer means is provided to monitor the pressure difference between the pressure for moving particles and the pressure for moving the sheathing liquid flow stream. Changes in differential pressure are automatically regulated to a preset difference. Means is included for providing a beam of light to illuminate the particles passing through the analysis region. Means detects light with respect to each moving particle and associates the detected light with one or more characteristics of each particle.

In accordance with the principles of the present invention, an automatic, electronic circuit with a transducer, a comparator, and a controller are associated with a housing for inclusion in a particle flow through apparatus such as flow cytometer. The automatically operating electronic circuit regulates the sample test tube air pressure used to transport particles into the passageway, including the capillary tube and the analysis orifice within the housing. The preferred embodiment provides compensation for the pressure drop due to the change in the reservoir liquid level during the operation of the cytometer.

This automatically operated control may also operate an air pump and/or regulators to supply required pressures which are necessary when debris clogs the filter. The feature of the present invention for accommodating varying liquid levels is easy to construct, simple to operate and primarily and directly regulates pressures applied to the sample test tube head space for controlling particle flow rate of a particle flow-through apparatus. By including the aforementioned monitoring and regulating features in the housing for a particle flow-through apparatus, the automatic system performs uniformly throughout cytometric study without manual readjustment.

The method for controlling the flow rate through a particle analysis apparatus includes transporting a sample of particles carried in a liquid by regulating air pressure applied to the liquid. The method further includes sensing the pressure difference between the air driving the sample and pressure of the liquid which ensheathes the sample. Thereafter, the method requires developing an electrical signal relative to the pressure difference sensed. An electrical reference signal of a pre-selected value relative to the desired sample flow rate is then provided. The electrical reference signal and the pressure difference signal are compared to generate an error signal. The error signal is then used to operate a pressure regulator.

Other advantages of the present invention will be perceived and understood by reading the detailed description which follows below.

BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1 is a perspective view of the preferred embodiment of a flow cytometer analyzer with an automatic flow control for use in determining one or more characteristics of particles or the like flowing in a liquid stream;

Figure 2 is a schematic illustration of typical elements and light paths of a flow cytometer embodying the sample flow control of the present invention;

Figure 3 is an enlarged cross-sectional view of the preferred flow housing of the present invention taken along line 3-3 of Figure 1;

Figure 4 is a schematic illustration of the relationship of the pressure control flow paths and the sample flow control including the connections of the control with the analyzer of Figure 1, and

Figure 5 is a block diagram schematic illustration of the sample flow control used in the analyzer of Figure 1 to regulate the flow rate of the particles through the analysis portion of the housing.

DETAILED DESCRIPTION

While this invention is satisfied by embodiments in many different forms, there is shown in the drawings and will herein be described in detail a preferred embodiment of the invention, with the understanding that the present disclosure is to be considered as exemplary of the principles of the invention and is not intended to limit the invention to the embodiment illustrated. The scope of the invention will be measured by the appended claims and their equivalents.

Referring to the drawings, and Figure 1 in particular, there is illustrated a flow cytometry apparatus 10 of the present invention for determining one or more characteristics of particles or the like. Apparatus 10, for example, can be a cell analyzer which includes a liquid sampling console 12 which is constructed to contain particle or cell detection and analysis elements as hereinafter described. In particular, analyzer 10 includes a liquid sampling console 12 which is constructed to contain the particle volume, light scatter and fluorescence measuring components, as hereinafter described, but which is separate from the analysis console 13. It will be pointed out hereinafter that analysis console 13 includes the electrical components, display screens and other data information regarding the

control and function of the analyzer apparatus 10. Liquid sampling console 12, as seen in Figure 1, includes a flow manifold assembly in the form of a housing 14 which is designed to provide a stream of flowing liquid containing the particle to be analyzed. In the apparatus being described, the particles for analysis may be included in a test tube 15 which can be removably positioned onto housing 14. Before describing the details of housing 14, a general description of the optical and flow elements of flow cytometry apparatus 10 will be provided.

Figure 2 is a schematic illustration of the general optical and flow elements embodied in a flow cytometer of the present invention. In addition to the general optical and flow elements of the apparatus to be described, other details of a cell analyzer apparatus useful in conjunction with the present invention are described in European Patent No. 0068404. It is understood that the housing 14 of the present invention is useful in many different types of flow cytometry or flow fluorometric equipment which measure light scatter, particle volume, fluorescence, or other optical parameters for the identification, quantification or enumeration of cells, particles or the like in a sample liquid medium. As illustrated in Figure 2, light energy is provided for the flow cytometer by a light source 16 such as a laser which provides a coherent beam of light at a singular wavelength or an arc lamp, such as a mercury or xenon arc lamp, which provides an incoherent beam of light comprising a broad spectrum of wavelengths.

Excitation energy is transmitted in the flow cytometer by a beam of light 18 produced by light source 16. Typically, the beam of light passes through focusing lens 19 which focuses the light beam at the liquid stream containing the particles or cells under investigation, and which will be described in more detail.

As each cell or particle passes through the focused light region where light beam 18 intersects the flowing liquid stream, light scattered by the cell or particle can be detected by an appropriate photodetector 21. Similarly, fluorescence, if emitted by particles energized by the illumination from the light source, can also be detected. Fluorescence emitted by autofluorescent particles or fluorescently labeled or stained particles in the liquid stream can be detected along the same axis as light beam 18 through lens 19, which may, for example, be a condenser lens assembly. This lens assembly is preferably, but not necessarily, an epi-illuminating system which uses the same lens for imaging excitation light and for receiving fluorescence emission from the particles. Fluorescence emitted by the flowing particles can be directed to a dichroic mirror 22 before being collected by fluorescence

detector 24. More than one fluorescence detector can be employed in order to detect fluorescence emitted from the particles at different wavelengths. Photodetector 21 and fluorescence detector 24 can be well-known photomultiplier tubes, or similar devices which convert light signals into electrical impulses, so that the light thereby detected can be associated with the fluorescently labeled cells and cells of a specific size flowing through the apparatus. The electrical signals from photodetector 21 and fluorescence detector 24 are typically fed to the electronics 28 of the apparatus for purposes of display 29, storage or further processing so that one or more characteristics of the cells or particles under analysis can be determined.

Turning now to Figure 3, the details of housing 14 of the present invention are more clearly illustrated. It can be seen that housing 14 includes a body member 30 which, in the embodiment being described, is preferably in the form of a block or rectangular prism. Although not shown in the drawings, the block form of housing 14 facilitates the mounting of the housing within the flow cytometer apparatus 10. Extending through housing 14 is a passageway 32 which is defined by three segments: an analysis portion 32a, a pre-analysis portion 32b, and a post-analysis portion 32c. As seen in Figure 3, the pre-analysis, analysis and post-analysis portions of passageway 32 lie on the same axis through body member 30 and are arranged in that order relative to the direction of particle flow through the passageway 32.

It is preferred that analysis portion 32a and post-analysis portion 32c of the passageway be cylindrically shaped bores extending into body member 30. On the other hand, it is preferred that pre-analysis portion 32b of the passageway be tapered so that it includes tapered walls 34 defining a frustoconical passageway having its narrow end facing toward analysis portion 32a of the passageway.

Preferably positioned within analysis portion 32a of the passageway is a flowcell or flow chamber 35 which facilitates the analysis of cells or particles under investigation. Flowcell 35 includes an orifice 36 which is preferably sized to permit the passage of substantially one particle at a time therethrough. As a light beam intersects the region defined by orifice 36, particles or cells which pass through the orifice also pass through the light beam thereby establishing a basis for a light-related signal which can be detected.

So that light energy can be available to illuminate the region defined by orifice 36 in the flowcell, body member 30 of the housing includes a recess 38 into which lens assembly 19 is positioned so that the lens assembly lies adjacent flowcell 35. This type of arrangement suggested by

the illustration in Figure 2 is consistent with a technique known as epi-illumination for providing light energy to interrogate the particles under analysis. Light is directed through lens assembly 19 at an angle substantially orthogonal to the aforementioned direction of particle flow through the flowcell. Lens assembly 19 can include one or more lenses in a condenser lens assembly for focusing incident light on the particles which pass through orifice 36, and can receive light such as fluorescence from the particles which have been illuminated by the incident light beam 18. Of course, the present invention contemplates that light from the particles can be detected in any direction with respect to the axis of the incident light beam. The appropriate light detectors are positioned at the desired angle for collecting light scattered or emitted by the particles or for detecting light energy absorbed by the particles. To this end, one or more windows 40 extend through body member 30 into flowcell 35 through which light passes for collection by the photodetector elements, see figure 3. On the other hand, it is not necessary to provide such a window if body member 30 is sufficiently light transmissive to allow light to pass therethrough in sufficient strength to be detected. It is, however, preferred that flowcell 35 be light transmissive and also that the flowcell be removable from body member 30 in the event that it needs cleaning, replacement or change.

Body member 30 also includes a first channel 42 which is in fluid communication with pre-analysis portion 32b of the passageway. Channel 42, in this embodiment, extends through a side block 44 of body member 30 so that this channel is substantially at right angles to the axis of passageway 32. Side block 44 includes a valve 45, or like device, which is operative to selectively open or close channel 42. Although not shown in Figure 3, valve 45 can be operated manually, electrically, pneumatically or any other convenient technique of operation. A fluid connector 46 is positioned on side block 44 so that its lumen 48 is in fluid communication with channel 42. It is the purpose of channel 42 to provide a passageway for the introduction of a liquid for sheathing particles which flow into analysis portion 32a of the passageway, and which more specifically flow through flowcell 35. The provision of a sheath liquid for a hydrodynamically focused fluid flow system is well-known in the art and is described in the mentioned patents. The sheath liquid is generally pressurized with air and typically flows through channel 42 at a pressure of between 0.5 and 10 psi and at a rate of 10 to 20 ml. per minute. The sheath liquid is usually a saline solution which is substantially particle free so that it does not interfere with the analysis.

Communicating with post-analysis portion 32c

of the passageway is another channel 50 which also extends through body member 30 in the embodiment being described. Second channel 50 also extends at substantially right angles to the axis of passageway 32. In fluid communication with channel 50 is a fluid connector 52 having a lumen 54 therethrough. It is the purpose of channel 50 to provide a passageway for the passage of particles and liquids out of housing 14 after passing through the analysis portion of the passageway. It can be seen that channel 50 has its interior end 55 preferably open to post-analysis portion 32c of the passageway.

Particles or cells to be analyzed are preferably transported through a hollow tube 58 with a lumen 59 extending therethrough. Tube 58 extends substantially along the axis of passageway 32 and has an inner end 60 positioned in pre-analysis portion 32b of the passageway. It is preferred that inner end 60 be positioned within tapered walls 34 of the pre-analysis portion so that the inner end 60 of the tube lies adjacent flowcell 35 in the analysis portion of the passageway. Tube 58 has its outer end 62 extending outwardly of body member 30. The body member of the housing preferably includes a circularly shaped extension 64 through which tube 58 extends before passing outwardly of the body member. A gasket 65, or other like element for providing a liquid-tight seal, is positioned around circularly shaped extension 64. It can be seen in Figure 3 that test tube 15 is positioned so that it fits onto extension 64 with gasket 65 facilitating a liquid-tight seal between the test tube and extension 64 of the body member. Test tube 15 includes sampling liquid 66 and particles 68 to be analyzed. Outer end 62 of the tube extends into sampling liquid 66 in this embodiment.

In order to cause particles 68 in the sampling liquid to be transported into tube 58, an annular passageway 70 is provided around the exterior surface of tube 58. This annular passageway includes an open end 71 surrounding tube 58 at the distal end of extension 64. A third channel 72 extends through body member 30 and is in fluid communication with annular passageway 70. A fluid connector 74 on the side of the body member includes a lumen 75 which is in fluid communication with channel 72. It is the purpose of connector 74 to be connected to a source of regulated pressurized air or other fluid to serve as a driving force of pressure into the test tube so that sampling liquid 66 and particles 68 may pass through lumen 59 of tube 58. Normally, the air is delivered through channel 72 at a slightly higher pressure than that applied to drive the sheath liquid through channel 42. In the preferred case, the regulated air pressure may be controlled at 5.0 psi or 4.0 psi for a selected high or low flow rate of 1.5 microliters

per second, or 0.25 microliters per second, respectively. Particles 68 pass out of the inner end of the tube into pre-analysis portion 32b of the passageway. Here, the particles and sampling liquid become ensheathed by the sheathing liquid so that the particles pass substantially one at a time through orifice 36 in flowcell 35, as seen in Figure 3. The confluence between the sampling liquid (and particles) and the sheath liquid form a coaxial, bi-component stream. The sampling liquid containing the particles 68 to be analyzed forms the inner component of the flowing stream. When the stream enters the flowcell 35, there is substantial equilibration in the velocities of the sheath liquid and the sample liquid and the particles are centered in the middle of the stream away from the walls of the flowcell.

Once in the flowcell, the particles can be interrogated by light which enters the flowcell through lens assembly 19 so that light-related information may be determined with respect to each particle. After the particles, sampling liquid and sheathing liquid pass through the analysis region of the passageway, flow continues through channel 50 for passage out of housing 14.

It is appreciated that the various air pressures and resulting flow rates could be manually adjusted by controls on the liquid sampling console. A typical sample flow rate is in the range of 0.25 to 1.5 microliters per second of sampling liquid through the sampling tube. Furthermore, the air pressure in channel 72 can be adjusted to control the count rate of particles through the flow chamber. Typically, the count rate would range between 100 and 1,000 particles per second flowing through the flow cell 35. The design of passageway 32 and the positioning of sample tube 58 therein is intended to offer minimal flow resistance to the bi-component stream of liquid as it flows toward flowcell 35.

In figure 4 there is a schematic illustration of the air pressure control 80 for the sample test tube 15, whereby the flow rate of particles 68 is regulated. The air pressure control 80 includes an air pump 82 connected to a pressure regulator 84 adjusted to provide a head pressure input of 4.5 psi to the sheath liquid supply reservoir 86. The outlet from reservoir 86 is connected to a filter 88 which removes any particulate matter from the sheath liquid as it is transported to the lumen 48 of side block 44. During use, the drop in sheath liquid level and pressure drop across filter 88 are not significant with regard to the flow rate of sheathing liquid at the pre-analysis portion 32b of passageway 32. The change in head pressure in reservoir 86 has been found to be less than 0.2 psi as the liquid level in the reservoir 86 drops from full toward empty.

Air pump 82, in figure 4, is also connected to

the sample flow control 90 for providing pressurized air to be used to drive the liquid 66 and particles 68 through tube 58 into pre-analysis portion 32b of housing 14. Sample flow control 90 regulates the air pressure applied to test tube 15 through lumen 75 so that the pressure is at 4 psi or 5 psi depending upon whether a low or a high flow rate of particles 68 is desired. Without regulation, the pressure in channel 42 can change significantly during a flow analysis as the liquid is driven from reservoir 86. A waste reservoir 92 is connected to lumen 54 to collect the liquids and particles after they have passed through the post-analysis portion 32c of the passageway 32.

The sample flow control 90 is shown in schematic block diagram form in figure 4; more details are illustrated in the schematic shown in figure 5. The sample flow rate setting switch 93 can be preset at a high flow rate setting (for liquid 66 and particles 68) of 1.5 microliters per second, or a low flow rate setting of 0.25 microliters per second as required for the sample to be tested by particle flow analysis. The switch 93 is shown connected to the high flow rate setting; a broken line illustrates the switch connection for the low setting.

The difference in the high and low setting for switch 93 is a change in preset reference voltages indicated as a positive electrical input signal for comparator 94. Comparator 94 in the preferred embodiment includes an operational amplifier in an integrator mode circuit whereby accurate control output signals are delivered for use in regulating the air pressure supplied to test tube 15 by way of the lumen 75. As can be seen in figure 5, the comparator 94 is connected electrically in circuit with an automatic pressure regulator 96 which is in the nature of an electrically operated valve. Regulator 96 includes a solenoid 98 having an electromagnetic coil 100 and a resiliently bias plunger 102 responsive to currents flowing through coil 100. The coil 100 is connected electrically to the output of the comparator 94 and in a known manner generates an electro-magnetic field responsive to the comparator output. Plunger 102 is normally biased or urged against a valve ball 104 which is held against a valve seat 106 to seal a bleed port 108 located therebeneath.

Air pressure from pump 82 through lumen 75 is regulated by ball 104 to maintain either the high or low particle flow rate setting by bleeding off excess air pressure when the ball 104 is allowed to unseat. In particular, the plunger 102 is drawn away from the ball 104 when the coil 100 is energized allowing air pressure between pump 82 and lumen 75 to decrease to the proper value. An air flow restrictor 110 is located in the air supply between the pump 82 and the valve seat 106, so that air available for bleeding through port 108 does not

exceed the capacity of air pump 82. The resilient bias of the plunger 102 is such that the regulator 96 does not become unstable and produces sufficiently high pressures in lumen 75 when coil 100 is not energized. Specifically, undesirable oscillation or sympathetic harmonics of the plunger are avoided by the proper selection of components so that the operation of the valve ball 104 is controlled. The air pressure applied to the test tube 15 via lumen 75 is thus modulated as required to produce the desired differential pressure.

The sample flow control 90 requires an input signal in order to stimulate comparator 94 into generating an appropriate output for activating solenoid 98 of the automatic pressure regulator 96. A differential pressure transducer 112 is used to convert the pressure difference between the sheathing liquid pressure monitored in lumen 48 and the air pressure in sample test tube 15 as measured in lumen 75, see figures 3, 4 and 5. The change in liquid pressure in lumen 48 is about 0.2 psi during operation of the apparatus 10 and that decrease is relatively insignificant. Throughout an analysis procedure differential pressure between lumen 48 and lumen 75 is about 1.2 psi when the analyzer apparatus 10 is set for the high particle flow rate and is approximately 0.2 psi when the apparatus 10 is set for the low particle flow rate. It should be appreciated that both the sheath liquid pressure and sample air pressure will decrease, but only the sample air pressure will decrease significantly.

Previous manual independent regulation of the air pressure required constant manual adjustment in order to maintain the preferred particle flow rate through orifice 36. The differential pressure transducer 112, in the preferred embodiment, includes a solid state strain gauge device having a silicon diaphragm with Wheatstone bridge circuitry which produces a changing signal as a functional of strain or load on the diaphragm. In this application, the saline sheathing liquid in lumen 48 pressurizes one side of the diaphragm and the sample air pressure in lumen 75 is against the other side.

As is known, the variations in liquid and air pressures will produce a signal which, in the preferred embodiment, is a voltage applied to the negative input of the comparator 94, as shown in figure 5. The varying voltage output of differential pressure transducer 112 is directly proportional to the change in the differential air and liquid pressures sensed in lumens 75 and 48, respectively. When the transducer voltage is compared to the (high or low) reference voltage, the resulting output directly controls bleed off of excess air pressure. The capacity of pump 82 is more than required whereby the air pressure in lumen 75 can be maintained at the required level for regulation of particle flow rate. While the liquid pressure in

lumen 48 does change somewhat, that change is sensed by the input to transducer 112 and is thus accounted for. The significant air pressure in lumen 75 is directly regulated by sample flow control 80.

Claims

1. A sample flow control for a flow cytometer comprising:

a body member having a passageway therethrough for the passage of particles which are to be analyzed, said passageway including an analysis portion, a pre-analysis portion and a post-analysis portion;

a differential pressure transducer in fluid communication with a sheath liquid supplied to the pre-analysis portion of said passageway and adapted for fluid communication with the test tube containing a sample of particles connected to the pre-analysis portion of said passageway for providing a differential pressure input signal equivalent to the pressure difference therebetween;

comparator means connected to receive said input signal and a preset reference signal for providing an operative control output comprising the difference between the signals; and

regulating means powered by said operative control output to maintain the pressure applied to the sample test tube for driving particle flow therefrom at a preestablished flow value.

2. The control of Claim 1 wherein the analysis portion of said passageway includes a flowcell having an orifice sized to permit the passage of substantially one particle at a time through said analysis portion, and wherein said regulating means includes an air pump operative to maintain the pressure applied to the sample test tube to drive particles through said orifice at the preestablished flow rate and wherein said body member includes means for permitting light to be directed at said orifice at an angle substantially orthogonal to the direction of particle flow through said flowcell.

3. The control of Claim 2 which further includes a sample tube having an inner end positioned in said pre-analysis portion of said passageway and an outer end extending outwardly of said body member into the sample test tube, said sample tube having a lumen extending therethrough for the passage of particles from said test tube toward the analysis portion of the passageway.

4. The control of Claim 3 wherein a pressure regulating transducer is connected to receive controlling input from said comparator means operative output for regulating an air supply to pressurize said sample test tube containing particles supplied through said lumen to said passageway pre-analysis portion.

5. A sample flow control for a particle-flow through apparatus having a supply of particles hydrodynamically focused within a sheathing liquid applied to said particles comprising:

5 a body member having a passageway therethrough including an analysis region through which substantially one particle at a time may pass in the direction of flow when said apparatus is operating, an operative means responsive to differential pressure in said passageway when the supply of particles to be analyzed and the sheathing liquid supply flow through said passageway, said operative means adjusting the force to cause the particles to flow through said analysis region.

10 15 20 25 30 35 40 45 50 55 60 65 70 75 80 85 90 95 100 105 110 115 120 125 130 135 140 145 150 155 160 165 170 175 180 185 190 195 200 205 210 215 220 225 230 235 240 245 250 255 260 265 270 275 280 285 290 295 300 305 310 315 320 325 330 335 340 345 350 355 360 365 370 375 380 385 390 395 400 405 410 415 420 425 430 435 440 445 450 455 460 465 470 475 480 485 490 495 500 505 510 515 520 525 530 535 540 545 550 555 560 565 570 575 580 585 590 595 600 605 610 615 620 625 630 635 640 645 650 655 660 665 670 675 680 685 690 695 700 705 710 715 720 725 730 735 740 745 750 755 760 765 770 775 780 785 790 795 800 805 810 815 820 825 830 835 840 845 850 855 860 865 870 875 880 885 890 895 900 905 910 915 920 925 930 935 940 945 950 955 960 965 970 975 980 985 990 995 1000 1005 1010 1015 1020 1025 1030 1035 1040 1045 1050 1055 1060 1065 1070 1075 1080 1085 1090 1095 1100 1105 1110 1115 1120 1125 1130 1135 1140 1145 1150 1155 1160 1165 1170 1175 1180 1185 1190 1195 1200 1205 1210 1215 1220 1225 1230 1235 1240 1245 1250 1255 1260 1265 1270 1275 1280 1285 1290 1295 1300 1305 1310 1315 1320 1325 1330 1335 1340 1345 1350 1355 1360 1365 1370 1375 1380 1385 1390 1395 1400 1405 1410 1415 1420 1425 1430 1435 1440 1445 1450 1455 1460 1465 1470 1475 1480 1485 1490 1495 1500 1505 1510 1515 1520 1525 1530 1535 1540 1545 1550 1555 1560 1565 1570 1575 1580 1585 1590 1595 1600 1605 1610 1615 1620 1625 1630 1635 1640 1645 1650 1655 1660 1665 1670 1675 1680 1685 1690 1695 1700 1705 1710 1715 1720 1725 1730 1735 1740 1745 1750 1755 1760 1765 1770 1775 1780 1785 1790 1795 1800 1805 1810 1815 1820 1825 1830 1835 1840 1845 1850 1855 1860 1865 1870 1875 1880 1885 1890 1895 1900 1905 1910 1915 1920 1925 1930 1935 1940 1945 1950 1955 1960 1965 1970 1975 1980 1985 1990 1995 2000 2005 2010 2015 2020 2025 2030 2035 2040 2045 2050 2055 2060 2065 2070 2075 2080 2085 2090 2095 2100 2105 2110 2115 2120 2125 2130 2135 2140 2145 2150 2155 2160 2165 2170 2175 2180 2185 2190 2195 2200 2205 2210 2215 2220 2225 2230 2235 2240 2245 2250 2255 2260 2265 2270 2275 2280 2285 2290 2295 2300 2305 2310 2315 2320 2325 2330 2335 2340 2345 2350 2355 2360 2365 2370 2375 2380 2385 2390 2395 2400 2405 2410 2415 2420 2425 2430 2435 2440 2445 2450 2455 2460 2465 2470 2475 2480 2485 2490 2495 2500 2505 2510 2515 2520 2525 2530 2535 2540 2545 2550 2555 2560 2565 2570 2575 2580 2585 2590 2595 2600 2605 2610 2615 2620 2625 2630 2635 2640 2645 2650 2655 2660 2665 2670 2675 2680 2685 2690 2695 2700 2705 2710 2715 2720 2725 2730 2735 2740 2745 2750 2755 2760 2765 2770 2775 2780 2785 2790 2795 2800 2805 2810 2815 2820 2825 2830 2835 2840 2845 2850 2855 2860 2865 2870 2875 2880 2885 2890 2895 2900 2905 2910 2915 2920 2925 2930 2935 2940 2945 2950 2955 2960 2965 2970 2975 2980 2985 2990 2995 3000 3005 3010 3015 3020 3025 3030 3035 3040 3045 3050 3055 3060 3065 3070 3075 3080 3085 3090 3095 3100 3105 3110 3115 3120 3125 3130 3135 3140 3145 3150 3155 3160 3165 3170 3175 3180 3185 3190 3195 3200 3205 3210 3215 3220 3225 3230 3235 3240 3245 3250 3255 3260 3265 3270 3275 3280 3285 3290 3295 3300 3305 3310 3315 3320 3325 3330 3335 3340 3345 3350 3355 3360 3365 3370 3375 3380 3385 3390 3395 3400 3405 3410 3415 3420 3425 3430 3435 3440 3445 3450 3455 3460 3465 3470 3475 3480 3485 3490 3495 3500 3505 3510 3515 3520 3525 3530 3535 3540 3545 3550 3555 3560 3565 3570 3575 3580 3585 3590 3595 3600 3605 3610 3615 3620 3625 3630 3635 3640 3645 3650 3655 3660 3665 3670 3675 3680 3685 3690 3695 3700 3705 3710 3715 3720 3725 3730 3735 3740 3745 3750 3755 3760 3765 3770 3775 3780 3785 3790 3795 3800 3805 3810 3815 3820 3825 3830 3835 3840 3845 3850 3855 3860 3865 3870 3875 3880 3885 3890 3895 3900 3905 3910 3915 3920 3925 3930 3935 3940 3945 3950 3955 3960 3965 3970 3975 3980 3985 3990 3995 4000 4005 4010 4015 4020 4025 4030 4035 4040 4045 4050 4055 4060 4065 4070 4075 4080 4085 4090 4095 4100 4105 4110 4115 4120 4125 4130 4135 4140 4145 4150 4155 4160 4165 4170 4175 4180 4185 4190 4195 4200 4205 4210 4215 4220 4225 4230 4235 4240 4245 4250 4255 4260 4265 4270 4275 4280 4285 4290 4295 4300 4305 4310 4315 4320 4325 4330 4335 4340 4345 4350 4355 4360 4365 4370 4375 4380 4385 4390 4395 4400 4405 4410 4415 4420 4425 4430 4435 4440 4445 4450 4455 4460 4465 4470 4475 4480 4485 4490 4495 4500 4505 4510 4515 4520 4525 4530 4535 4540 4545 4550 4555 4560 4565 4570 4575 4580 4585 4590 4595 4600 4605 4610 4615 4620 4625 4630 4635 4640 4645 4650 4655 4660 4665 4670 4675 4680 4685 4690 4695 4700 4705 4710 4715 4720 4725 4730 4735 4740 4745 4750 4755 4760 4765 4770 4775 4780 4785 4790 4795 4800 4805 4810 4815 4820 4825 4830 4835 4840 4845 4850 4855 4860 4865 4870 4875 4880 4885 4890 4895 4900 4905 4910 4915 4920 4925 4930 4935 4940 4945 4950 4955 4960 4965 4970 4975 4980 4985 4990 4995 5000 5005 5010 5015 5020 5025 5030 5035 5040 5045 5050 5055 5060 5065 5070 5075 5080 5085 5090 5095 5100 5105 5110 5115 5120 5125 5130 5135 5140 5145 5150 5155 5160 5165 5170 5175 5180 5185 5190 5195 5200 5205 5210 5215 5220 5225 5230 5235 5240 5245 5250 5255 5260 5265 5270 5275 5280 5285 5290 5295 5300 5305 5310 5315 5320 5325 5330 5335 5340 5345 5350 5355 5360 5365 5370 5375 5380 5385 5390 5395 5400 5405 5410 5415 5420 5425 5430 5435 5440 5445 5450 5455 5460 5465 5470 5475 5480 5485 5490 5495 5500 5505 5510 5515 5520 5525 5530 5535 5540 5545 5550 5555 5560 5565 5570 5575 5580 5585 5590 5595 5600 5605 5610 5615 5620 5625 5630 5635 5640 5645 5650 5655 5660 5665 5670 5675 5680 5685 5690 5695 5700 5705 5710 5715 5720 5725 5730 5735 5740 5745 5750 5755 5760 5765 5770 5775 5780 5785 5790 5795 5800 5805 5810 5815 5820 5825 5830 5835 5840 5845 5850 5855 5860 5865 5870 5875 5880 5885 5890 5895 5900 5905 5910 5915 5920 5925 5930 5935 5940 5945 5950 5955 5960 5965 5970 5975 5980 5985 5990 5995 6000 6005 6010 6015 6020 6025 6030 6035 6040 6045 6050 6055 6060 6065 6070 6075 6080 6085 6090 6095 6100 6105 6110 6115 6120 6125 6130 6135 6140 6145 6150 6155 6160 6165 6170 6175 6180 6185 6190 6195 6200 6205 6210 6215 6220 6225 6230 6235 6240 6245 6250 6255 6260 6265 6270 6275 6280 6285 6290 6295 6300 6305 6310 6315 6320 6325 6330 6335 6340 6345 6350 6355 6360 6365 6370 6375 6380 6385 6390 6395 6400 6405 6410 6415 6420 6425 6430 6435 6440 6445 6450 6455 6460 6465 6470 6475 6480 6485 6490 6495 6500 6505 6510 6515 6520 6525 6530 6535 6540 6545 6550 6555 6560 6565 6570 6575 6580 6585 6590 6595 6600 6605 6610 6615 6620 6625 6630 6635 6640 6645 6650 6655 6660 6665 6670 6675 6680 6685 6690 6695 6700 6705 6710 6715 6720 6725 6730 6735 6740 6745 6750 6755 6760 6765 6770 6775 6780 6785 6790 6795 6800 6805 6810 6815 6820 6825 6830 6835 6840 6845 6850 6855 6860 6865 6870 6875 6880 6885 6890 6895 6900 6905 6910 6915 6920 6925 6930 6935 6940 6945 6950 6955 6960 6965 6970 6975 6980 6985 6990 6995 7000 7005 7010 7015 7020 7025 7030 7035 7040 7045 7050 7055 7060 7065 7070 7075 7080 7085 7090 7095 7100 7105 7110 7115 7120 7125 7130 7135 7140 7145 7150 7155 7160 7165 7170 7175 7180 7185 7190 7195 7200 7205 7210 7215 7220 7225 7230 7235 7240 7245 7250 7255 7260 7265 7270 7275 7280 7285 7290 7295 7300 7305 7310 7315 7320 7325 7330 7335 7340 7345 7350 7355 7360 7365 7370 7375 7380 7385 7390 7395 7400 7405 7410 7415 7420 7425 7430 7435 7440 7445 7450 7455 7460 7465 7470 7475 7480 7485 7490 7495 7500 7505 7510 7515 7520 7525 7530 7535 7540 7545 7550 7555 7560 7565 7570 7575 7580 7585 7590 7595 7600 7605 7610 7615 7620 7625 7630 7635 7640 7645 7650 7655 7660 7665 7670 7675 7680 7685 7690 7695 7700 7705 7710 7715 7720 7725 7730 7735 7740 7745 7750 7755 7760 7765 7770 7775 7780 7785 7790 7795 7800 7805 7810 7815 7820 7825 7830 7835 7840 7845 7850 7855 7860 7865 7870 7875 7880 7885 7890 7895 7900 7905 7910 7915 7920 7925 7930 7935 7940 7945 7950 7955 7960 7965 7970 7975 7980 7985 7990 7995 8000 8005 8010 8015 8020 8025 8030 8035 8040 8045 8050 8055 8060 8065 8070 8075 8080 8085 8090 8095 8100 8105 8110 8115 8120 8125 8130 8135 8140 8145 8150 8155 8160 8165 8170 8175 8180 8185 8190 8195 8200 8205 8210 8215 8220 8225 8230 8235 8240 8245 8250 8255 8260 8265 8270 8275 8280 8285 8290 8295 8300 8305 8310 8315 8320 8325 8330 8335 8340 8345 8350 8355 8360 8365 8370 8375 8380 8385 8390 8395 8400 8405 8410 8415 8420 8425 8430 8435 8440 8445 8450 8455 8460 8465 8470 8475 8480 8485 8490 8495 8500 8505 8510 8515 8520 8525 8530 8535 8540 8545 8550 8555 8560 8565 8570 8575 8580 8585 8590 8595 8600 8605 8610 8615 8620 8625 8630 8635 8640 8645 8650 8655 8660 8665 8670 8675 8680 8685 8690 8695 8700 8705 8710 8715 8720 8725 8730 8735 8740 8745 8750 8755 8760 8765 8770 8775 8780 8785 8790 8795 8800 8805 8810 8815 8820 8825 8830 8835 8840 8845 8850 8855 8860 8865 8870 8875 8880 8885 8890 8895 8900 8905 8910 8915 8920 8925 8930 8935 8940 8945 8950 8955 8960 8965 8970 8975 8980 8985 8990 8995 9000 9005 9010 9015 9020 9025 9030 9035 9040 9045 9050 9055 9060 9065 9070 9075 9080 9085 9090 9095 9100 9105 9110 9115 9120 9125 9130 9135 9140 9145 9150 9155 9160 9165 9170 9175 9180 9185 9190 9195 9200 9205 9210 9215 9220 9225 9230 9235 9240 9245 9250 9255 9260 9265 9270 9275 9280 9285 9290 9295 9300 9305 9310 9315 9320 9325 9330 9335 9340 9345 9350 9355 9360 9365 9370 9375 9380 9385 9390 9395 9400 9405 9410 9415 9420 9425 9430 9435 9440 9445 9450 9455 9460 9465 9470 9475 9480 9485 9490 9495 9500 9505 9510 9515 9520 9525 9530 9535 9540 9545 9550 9555 9560 9565 9570 9575 9580 9585 9590 9595 9600 9605 9610 9615 9620 9625 9630 9635 9640 9645 9650 9655 9660 9665 9670 9675 9680 9685 9690 9695 9700 9705 9710 9715 9720 9725 9730 9735 9740 9745 9750 9755 9760 9765 9770 9775 9780 9785 9790 9795 9800 9805 9810 9815 9820 9825 9830 9835 9840 9845 9850 9855 9860 9865 9870 9875 9880 9885 9890 9895 9900 9905 9910 9915 9920 9925 9930 9935 9940 9945 9950 9955 9960 9965 9970 9975 9980 9985 9990 9995 9999

comprising;
means for moving particles in a liquid flow stream;
a body member having a passageway therethrough
including an analysis region through which said
moving particles pass substantially one at a time in
the direction of flow;
operative means responsive to pressure changes
while driving particles through said passageway
and the pressure of sheathing liquid flowing
through said analysis region;
means for providing a beam of light to illuminate
said particles passing through said analysis region;
and
means for detecting light with respect to each
moving particle and for associating said detected
light with one or more characteristics of each par-
ticle.

9. A method for controlling the flow rate
through a particle analysis apparatus transporting a
sample of particles carried in a liquid by regulating
air pressure applied to the liquid including the
following steps;

sensing a pressure difference between air driving a
sample of particles and liquid to ensheathe the
sample,

developing an electrical signal relative to the pres-
sure difference sensed,

providing an electrical reference signal of a pre-
selected value relative to a desired sample flow
rate,

comparing the electrical reference signal and the
pressure difference signal to generate an error sig-
nal,

using the error signal to operate a pressure regula-
tor, and

effecting a control function of the pressure regula-
tor for at least one of the differential pressures
sensed.

10. The method of Claim 9 wherein the step of
effecting control includes biasing an operator used
for control to provide stable regulation and wherein
the step of providing at least two reference signals
permits the selection of two or more sample flow
rates.

5

10

15

20

25

30

35

40

45

50

55

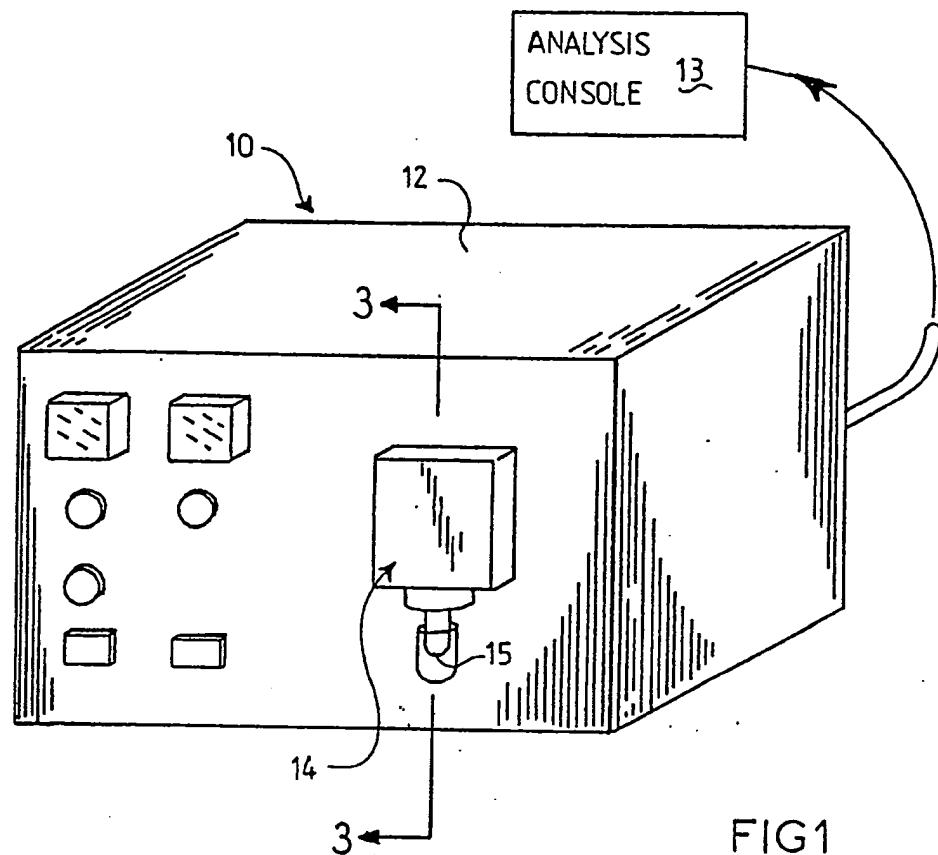


FIG1

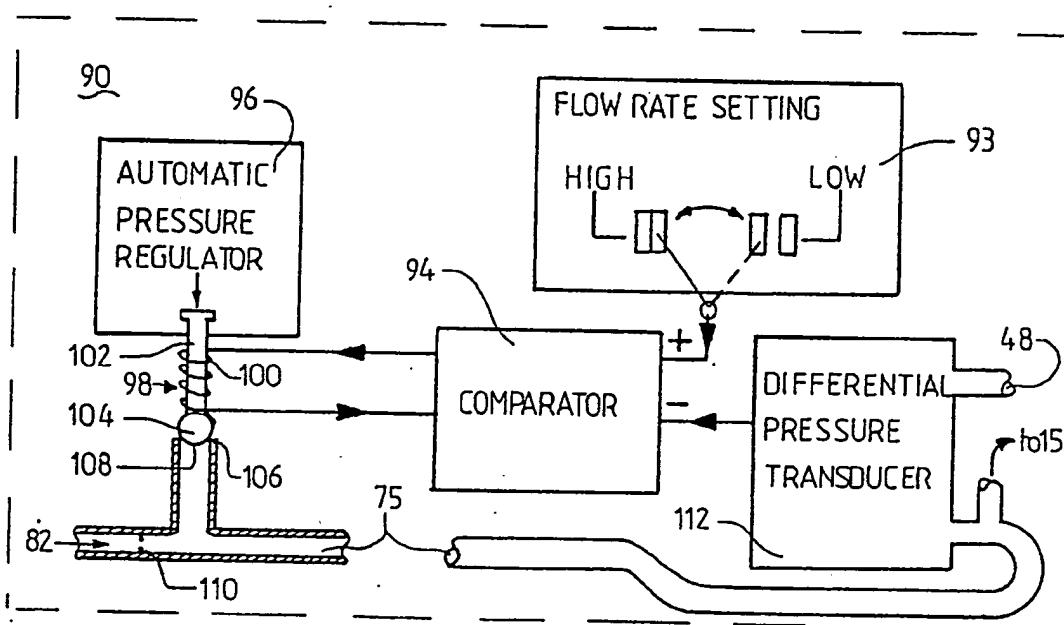


FIG5

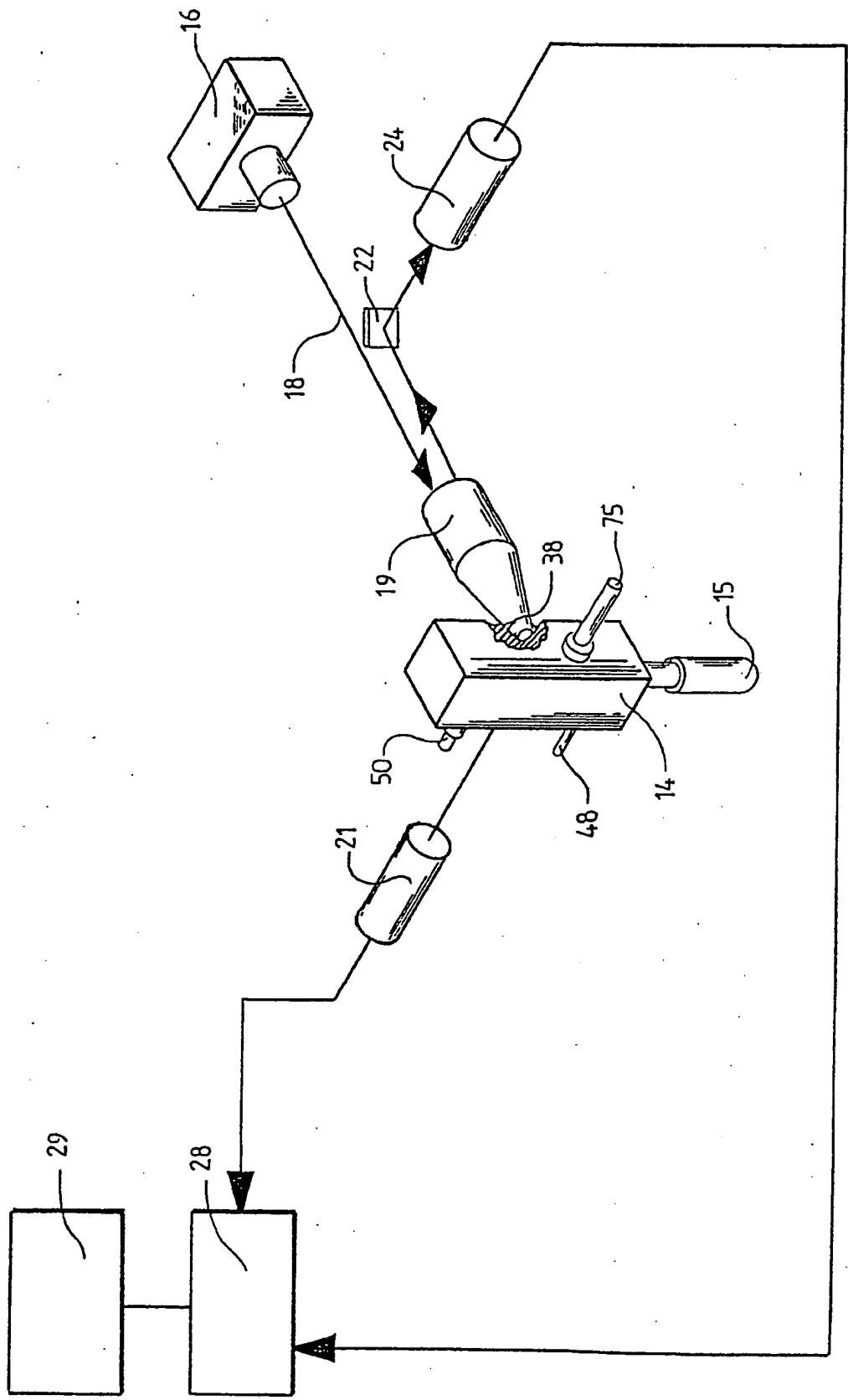


FIG 2

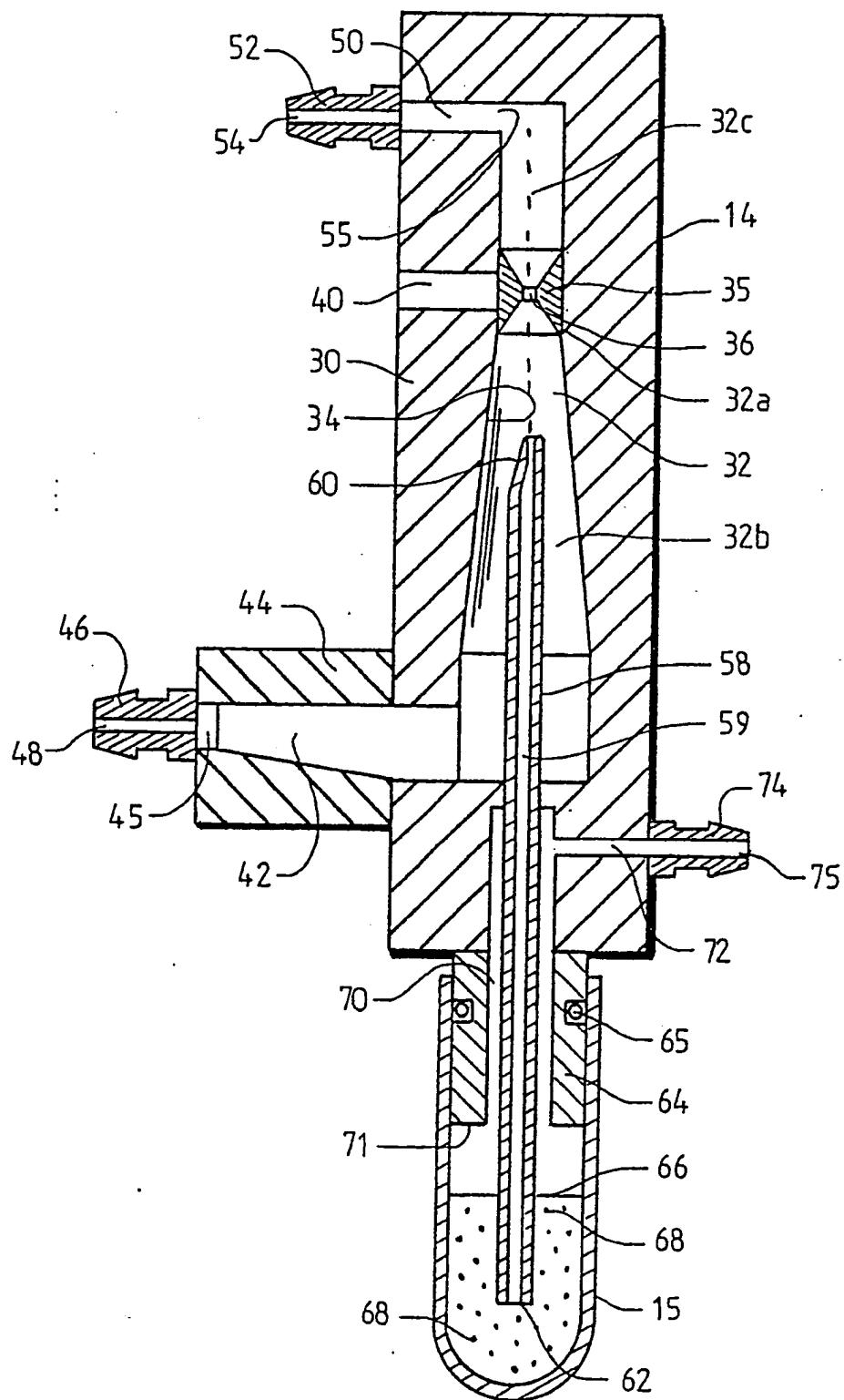


FIG 3

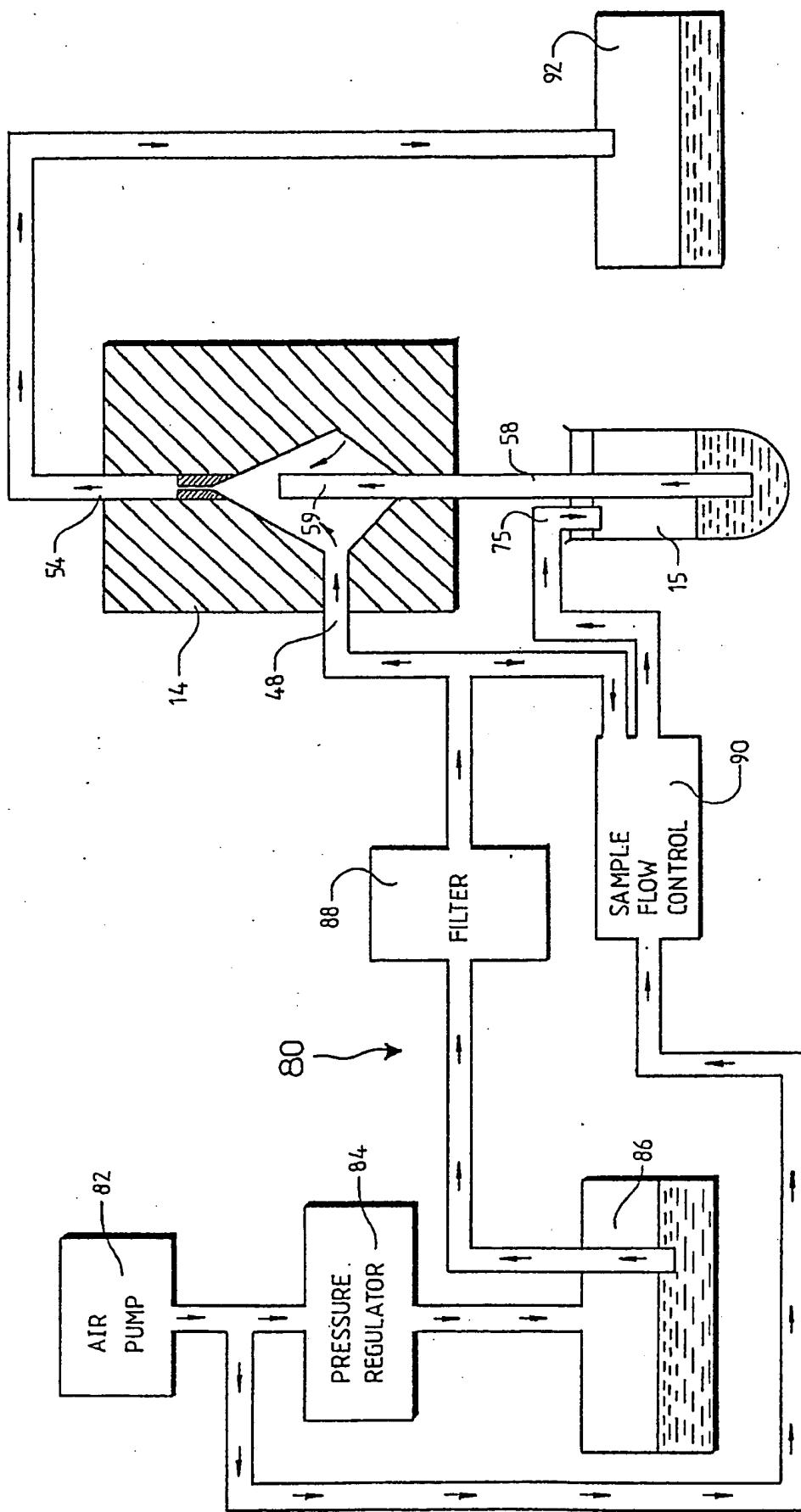


FIG 4